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Title: Sample Receipt and Wash Procedure for Celery		
Revision: 1	Replaces: 10/01/01	Effective: 02/01/02

1. <u>Purpose</u>:

To provide standard procedures for receipt and washing of fruit and vegetable samples for the USDA/AMS Microbiological Data Program (MDP).

2. Scope:

This standard operating procedure (SOP) shall be followed by all laboratories conducting microbiological studies for MDP, including support laboratories conducting non-routine activities that may impact the program.

3. Outline of Procedure:

- 5.1 Equipment and Materials
- 5.2 Media and Reagents
- 5.3 Definition of Sample
- 5.4 Receipt of Samples
- 5.5 Wash Method

4. <u>References</u>:

- Work plan for Microbiological Data Program Pilot Study, August 25, 1999.
- SAMP-PROC-2, MDP Sampling Procedures on Site.
- SAMP-PROC-3, Packing and Shipment of MDP Samples.
- Sadovski, A.Y.; Fattal, B.; Goldberg, D. *Microbial Contamination of Vegetables Irrigated with Sewage Effluent by the Drip Method.* Journal of Food Protection, Vol. 41, No. 5, 336-340, May 1978.
- FDA Survey of Domestic Fresh Produce, U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, May 10, 2000.

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5. **Specific Procedures:**

5.1 Equipment and Materials

- a Balance, minimum 1000 g with minimum of 0.1 g sensitivity
- b. Rotary shaker, set at 2.54 cm (1 inch) stroke
- c. Plastic bags, sterile, suitable size to hold sample and diluent. e.g., sterile 3500 stomacher bag
- d. Forceps, tongs, slotted spoons, sterile
- e. Thermometer, Raytek Portable IR Sensor, P/N Rayst20CRUS
- f. Gloves, sterile

5.2 Media and Reagents

- a. Butterfield's phosphate diluent
- b. Tween 80

5.3 Definition of Sample

- a. When produce is taken from a terminal market warehouse, or other primary source, the commodities taken (usually 3 per produce type) are referred to as a "site sample."
- b. Upon arrival at the laboratory, all units in a "site sample" are referred to as a "laboratory sample." For the purposes of the laboratory SOPs, the word sample will refer to the individual "laboratory samples."
- c. There are usually three "laboratory samples" in each "commodity site sample."

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5.4 Receipt of Samples

- a. Upon arrival at the laboratory, samples will be logged in, recording the temperature of one sample taken at the warehouse, and the temperature will be taken of all three samples, along with the date and time of receipt noted and recorded on the laboratory work sheet. The temperature of the produce will be determined by pointing and activating the IR thermometer at the surface of one of each type of sample. **Do not** take the temperature through the plastic bag. Carefully open a sample bag to obtain direct access to the surface of the sample. The bags should be sealed in such a way that they can be opened and re-sealed easily. If this is not the case, contact your sampling manager to arrange for appropriate modifications in bag closure procedures. Take the temperature of each sample. Test all laboratory samples regardless of temperature unless spoilage has taken place.
- b. The samples will be refrigerated until analysis begins. The analysis shall be performed as soon as realistically possible but is not to exceed 24 hours after receipt in the laboratory. Record the holding time of sample, i.e., the time between receipt and the time testing starts.
- c. The samples will be analyzed using prescribed methods as specified by the MDP Program Manager. All analyses will be started from the sample wash diluent. The laboratory will run a media control and a positive and negative control for each analysis on each day a sample is analyzed.
- d. The laboratory will receive 3 samples (or multiples thereof) of the same produce in each shipping container. Each of the three celery samples shall be prepared as follows.
- e. Preparation of samples.
 - 1. The laboratory will test whole celery stalks. The sample from celery will be taken by aseptically pulling whole stalks of celery from the

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base of the bunch of celery (include leaves, if present). Enough stalks will be selected to obtain approximately 200 g per sample.

2. Test only whole stalks of celery. Do not reject celery stalks with slits. **Do not** test stalks of samples that show spoilage or are severely damaged.

5.5 Wash Method

- a. The analysis shall be performed as soon as realistically possible but is not to exceed 24 hours after receipt in the laboratory.
- b. All manipulations must be performed using sterile technique.
- c. Prepare sterile Butterfield's phosphate diluent plus 1% Tween 80 (v/v).
 - 1. If pre-sterilized Butterfield's phosphate diluent is used, the Tween 80 may be added as follows:
 - a) Place 100 mL of Tween 80 into a container and subject to flowing steam three times over three days at 100°C for a minimum of 20 minutes each day. Submerge only the tip of the pipette into the Tween 80 when transferring to Butterfield's phosphate buffer. Flush pipette three times after delivery.
 - 2. If Butterfield's phosphate diluent is prepared with the Tween 80 added prior to sterilization, no pretreatment of the Tween 80 is necessary.
- d. Tare a sterile bag.
- e. Add approximately 200 g of sample to bag. **There is to be no compositing of samples.** Each sample is tested individually.

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- f. Add an equal weight of diluent to the bag (+ 5 g). Seal the bag.
- g. Place the bag on a rotating shaker in such a way that the bag lies as flat as possible without leaking. Shake the sample at 160 rpm, stroke size 2.54 cm (1 inch) for 7 minutes. Turn bag over after half the shaking time has passed.
- h. Use the wash diluent as the sample.
- i. When calculating, use 1 mL of wash diluent as if it is 1 g of sample.
- j. Discard excess wash diluent and the original sample after testing begins.

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• Changed maximum laboratory holding time prior to analysis from 72 hours to 24 hours in subsections 5.4.b and 5.5.a